Effects of phytophogenic feed additives on bird health

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From January 1st 2006 onwards in the European Union (EU) antibiotics were banned as growth promoting feed additives. As the first and currently single Asian country the Republic of South Korea followed the EU example in 2011.

It can be expected that numerous further big countries in the world will also remove feed antibiotics within the next few years.

The protection of consumers’ health is the main reason for these decisions.

However, the removal of antibiotics at the same time means a big challenge, since alternative substances should be comparably effective with regard to their impact on the productivity of farm animals and with regard to the preservation of animal health.

Promising alternative

Within the alternatives to antibiotics, phytophogenic substances represent the most promising class of feed additives. This opinion is based on the availability of an infinite number of available plants with highly active ingredients. The spectrum of effects mediated by plant derived compounds includes the disturbance of microbial communication, antioxidant properties, and anti-inflammatory effects.

In this article, two aspects of selected phytophogenic compounds are looked at in more detail:

- Disturbance of microbial communication.
- Antioxidant and anti-inflammatory effects.

With regard to the efficiency to protect farm animals against disorders caused by pathogenic microorganisms, it has been frequently postulated, that phytophogenic substances (in particular essential oils and their compounds) exert bactericidal effects. By definition the term ‘minimum inhibitory concentration’ (MIC) towards a micro-organism (MO) means a reduction of MO viability by more than 90%.

The minimum bactericidal concentration (MBC) means a reduction of viability by more than 99%.

Tables 1 and 2 give an overview of the MIC concentrations of selected essential oils and essential oil compounds against several micro-organisms.

Neglecting an additional dilution effect of the feed in the intestine, the MIC concentrations give evidence that genuine bactericidal effects of phytophogenic compounds in the animal cannot be obtained with phytophogenic additives. Essential oil concentrations in the feed, unrolling bactericidal effects, would:

- Result in a reduced feed intake and performance due to the strong taste of the oils.
- Be economically unmanageable.

Quorum sensing

Quorum sensing (QS), or bacterial cell-to-cell communication, is a mechanism of gene regulation in which bacteria coordinate the expression of certain genes in response to the presence of small signalling molecules (inducers). This regulatory mechanism has been shown to control virulence gene expression in many different pathogens. Virulence factors include gene products involved in motility, adhesion to the host’s intestinal epithelium, host tissue degradation, iron acquisition, and toxin production.

The abundance of the signalling molecules in the environment thereby directly reflects the bacterial population density. If a certain threshold of inducer concentration is reached, the bacteria start to produce virulence factors, leading to the outbreak of the disease in the host.

Table 1. MIC values of selected single compounds from essential oils (Burt, 2004).

<table>
<thead>
<tr>
<th>Essential oil component</th>
<th>Bacteria species</th>
<th>MIC (estimated range) µL/mL ~ µg/mL ~ g/L ~ kg/t</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>Salmonella typhimurium</td>
</tr>
<tr>
<td>Carvacrol (Oregano)</td>
<td>0.25-5.0</td>
<td>0.22-0.25</td>
</tr>
<tr>
<td>Thymol (Thyme)</td>
<td>0.22-0.45</td>
<td>0.07</td>
</tr>
<tr>
<td>Citral (Lemon)</td>
<td>0.55</td>
<td>0.5</td>
</tr>
<tr>
<td>Eugenol (Clove)</td>
<td>1.0</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Table 2. MIC values of selected single compounds from essential oils (Burt, 2004).

<table>
<thead>
<tr>
<th>Essential oil component</th>
<th>Bacteria species</th>
<th>MIC (estimated range) µL/mL ~ µg/mL ~ g/L ~ kg/t</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>Salmonella typhimurium</td>
</tr>
<tr>
<td>Rosemary</td>
<td>4.5-10.0</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Oregano</td>
<td>0.50-1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Thyme</td>
<td>0.45-1.25</td>
<td>0.45-20</td>
</tr>
<tr>
<td>Sage</td>
<td>3.5-5.0</td>
<td>10.0-20.0</td>
</tr>
<tr>
<td>Clove</td>
<td>0.40-2.5</td>
<td>&gt;20.0</td>
</tr>
</tbody>
</table>

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In the context of QS by phyto- 
genic substances it could be shown that clove oil strongly inhibited viola- 
cin fluorescence in Chromobac- 
terium violaceum at already sub-
inhibitory concentrations (Table 3).

Promising results for QS by essen- 
tial oils were also reported for 
Campylobacter jejuni and the 
enterohemorrhagic E. coli strain 
O157:H7.

In the first mentioned study, 
already 0.10mmol/L carvacrol, cor- 
responding to 15mg carvacrol/L, 
significantly inhibited the motility of 
Campylobacter jejuni. In the latter 
study, 1mmol/L carvacrol 
(150mg/L) induced a strong heat-
shock response in E. coli and inhib-
ited flagellin synthesis, essential for 
the motility of the pathogen.

These results impressively show 
that phytoconstituents have the 
potential to disturb pathogenic 
micro-organisms very well.

In the future it is therefore of sig-
ificant interest to force research 
into studying the efficiency of phyto-
genic substances on QS of patho-
genic bacteria.

In the ‘post-antibiotic-era’ the dis-
ruption of QS of pathogenic bacteria 
by phytoconstituents represents 
one core strategy to preserve farm 
animals from infectious diseases of 
the intestine.

Additives and inflammation

Due to the ban of growth promot-
ing antibiotics, the infection of farm 
animals with species specific patho-
genic micro-organisms represents an 
issue of growing importance. In 
numerous cases, the infection with 
pathogens, does not result in the full 
blown clinical picture, but leads to a 
severe reduction of performance 
and causes economic damage.

This aspect is well known with 
regard to the infection of chickens 
with Eimeria tenella. Irrespective of 
its degree, an infection with intesti-
nal pathogens induces an inflammat-
ory response in the hosts.

During an infection with patho-
genic micro-organisms, intestinal 
cells secrete a variety of cytokines in 
order to attract cells of the immune 
system. In the early stages of 
immune response, macrophages, 
entering the affected tissues, pro-
duce a strong inflammatory reaction.

In later stages T cells are also 
involved in the promotion of inflam-
mation. The most important intra-
cellular transcription factor, 
triggering inflammation in a cell is the 
Nuclear factor ‘kappa-light-chain-
erythroid-derived 2’-like 2’ 
(NFkB).

NFkB on the one hand induces the 
synthesis of cytokines, responsible for 
the further recruitment and 
attachment of immune cells (for 
example. IL6, VCAM, ICAM) and of 
Cyclooxygenase 2 (COX2), produc-
ing pro-inflammatory prostaglandins.

On the other hand, NFkB action is 
needed for the production of anti-
inflammatory cytokines, responsible 
for the termination of an inflamma-
tion (for example IL-10), and of 
antioxidant enzymes, which enable 
cell survival and help to terminate an 
inflammation.

The endogenous antioxidant 
enzymes, which are of particular 
interest for the termination of an inflam-
amation are NAD(P)H-
Quinone-Oxidoreductase 
I (NQO1), Hem oxygenase 1 
(HO1) and glutathione peroxidase 2 
(GPx2). A common feature of these 
mentioned antioxidant enzymes 
consists in their specific synthesis 
mechanism. Their transcription and synthesis 
takes place, due to the release of the 
transcription factor ‘Nuclear fac-
tor (erythroid-derived) 2-like 2’ 
(Nrf2). In this context it must be 
mentioned that besides NFkB and 
oxidative stress, numerous phyto-
genic substances act as very strong 
Nrf2-releasing agents. Curcumin 
from curcuma longa and the brassi-
daceae isothiocyanate sulforaphane 
are the best characterised inducers of 
endogenous antioxidant enzymes 
by triggering Nrf2-release.

The induction of endogenous 
antioxidant enzymes concomitantly 
can reduce the severity of inflammat-
ion. In this context it could be 
shown that the induction of GPx2 
reduces COX2-dependent 
prostaglandin E2 synthesis.

The results of our own investiga-
tions with growing broilers revealed a 
considerable induction of a broad 
panel of Nrf2-dependent antioxidant 
enzymes and of phase II enzymes in 
the jejunum, by supplementing the 
diets with 150g/t turmeric oil or 
with sulforaphane-containing broc-
coli extract. In an own rat study it 
could be demonstrated that 
sulforaphane feeding significantly 
induced intestinal antioxidant 
enzymes, while reducing the expres-
sion of COXI, COXII, VCAM, and 
Monocyte Chemotactic Protein 1 
(MCP1).

In a recent study with Eimeria 
tenella infected broilers, the addition 
of capsicum and turmeric oleoresins 
to the diets strongly reduced intesti-
 nal lesion score and the expression 
levels of main pro-inflammatory 
cytokines.

The results of these and numerous 
other trials indicate that various 
phytoconstituents have promis-
ing effects with regard to the reduc-
tion of pathogen-induced intestinal 
inflammations in farm animals. In the 
future, research into the screening 
of anti-inflammatory phytoconstitu-
tes should be focused in order to 
develop phytoconstituents with cus-
tomised effects against species specific 
infectious diseases.

Moreover, there is a need to 
develop products with an increased 
content of active substances for the 
treatment of acute intestinal inflam-
ination.

**Conclusions**

- Due to their content of an infinite 
  variety of active ingredients, phyto-
genic substances represent one of 
  the most interesting and important 
  classes of current and future feed 
  additives.
- Phytoconstituents bear the 
  potential to effectively repress the 
  pathogenicity of intestinal micro-
  organisms and to prevent and treat 
  infectious diseases of farm animals.
- In the future a broad-based 
  research is needed to make the full 
  potential of phytoconstituents 
  usable in order to preserve the 
  health of farm animals and 
  consumers.

**References**

- Khan MS, Zahir M, Hasan S, 
  Husain FM, Ahmad I. (2009) 
  Inhibition of quorum sensing regulated 
  bacterial functions by plant essential 
  oils with special reference to clove oil. 

**Fig. 1. Interfaces between inducible antioxidant enzymes and immune response.**

**Table 3. Cell viability and quorum sensing inhibition of CV, incubated with clove oil (compiled from: Khan 
MS, Zahir M, Hasan S, Husain FM, Ahmad I.)**